Evidence-based Practice Center Systematic Review Protocol

Project Title: Clostridium difficile Infection Update

I. Background and Objectives for the Systematic Review

In December 2011 the Agency for Healthcare Research and Quality (AHRQ) published the results of Comparative Effectiveness Review (CER) No. 31, Effectiveness of Early Diagnosis, Prevention, and Treatment of *Clostridium difficile* Infection, prepared by the Minnesota Evidence based Practice Center.[1] This CER examined the evidence on the sensitivity and specificity of *C. difficile* infection laboratory diagnostic tests, the effectiveness of prevention strategies, and the effectiveness and harms of antibiotic and adjuvant treatments for adults with Clostridium difficile infection (CDI). In January 2014 AHRQ published a surveillance report that assessed whether an update of CER No. 31 was warranted. The report found new evidence for all key questions, suggesting the results were out of date.[2]

C. difficile Background

CDI rates in the United States (and globally) have increased in the last decade, along with associated morbidity and mortality. *Clostridium difficile* is a gram-positive, anaerobic bacterium that is generally acquired through ingestion. Various strains of the bacteria may produce disease generating enterotoxin A and cytotoxin B, as well as the lesser understood binary toxin. Use of the term CDI indicates the major focus of this review is on the presence of clinical disease, not asymptomatic carriage of *C. difficile*. CDI symptoms can range from mild diarrhea to severe cases including pseudomembranous colitis and toxic megacolon and death. Mortality from CDI is estimated at 2.4 deaths per 100,000 population in 2011.[3,4] Between 1999 and 2008 the mortality rate from CDI rose each year to a peak of 2.4 deaths per 100,000 in 2008, leveling off at 2.2 in 2009 and 2010, and rising again to 2.4 in 2011.

Distribution of CDI in the population is bimodal, with the largest incidence in elderly individuals, and a considerably smaller peak in children under age 10. The vast majority of severe morbidity and mortality is experienced in the elderly population.[5,6] In 2011 93 percent of deaths from *C. difficile* occurred in persons ≥65 years of age, the 17th leading cause of death in this age group.[4] Residents of long-term care facilities are at high risk, with up to half of health-care associated CDI cases beginning in long term care.[7] Incidence rates may increase by four- or five-fold during outbreaks.[8] Community associated CDI rates are generally lower, accounting for 27 percent of cases in a recent prevalence study,[9] but is also on the rise.[8]

New, more virulent strains have emerged since 2000, which affect a wider population, often with a lack of standard risk profiles such as previous hospitalization or antibiotic use, including children, pregnant women, and other healthy adults.[10] The hypervirulent strain accounts for 51 percent of CDI, compared to only 17 percent of historical isolates.[11,12] The time from symptom development to septic shock may be reduced in the hypervirulent strains, making quick diagnosis and proactive treatment regimens critical for positive outcomes.

Not all people who acquire *C. difficile* necessarily develop CDI. The likelihood of developing CDI is dependent on a number of factors that allow colonization and toxin production, including failure of the immune defenses and use of antibiotics, particularly broad-

Source: www.effectivehealthcare.ahrq.gov
Published online: December 23, 2014

spectrum or multiple antibiotics. In addition to eliminating, where possible, the offending antibiotic, and environmental and infection control strategies, recent prevention efforts at the patient level have also focused on improving immune defenses through healthy digestive function and gut flora, and nutritional status.[13] Other risk factors include increasing age, comorbidities, and use of gastric acid suppressant medications.[14] Mortality is associated with age, white blood cell count, serum albumin, and serum creatinine.[14] Risk profiles for recurrent CDI are similar.[15] One study which statistically modeled CDI within the hospital setting suggested that reducing patient susceptibility to infection is more effective in reducing CDI cases than lowering transmission rates.[16] Prevention measures, then, can target reducing both patient susceptibility to infection and the spread of the bacteria or spores.

Effective prevention of transmission and treatment of CDI is dependent on accurate diagnosis with short turn-around time. There are increasing numbers of diagnostic tests designed to detect either the presence of the organism, or toxins A and/or B, with a variety of sensitivities, specificities, predictive values, biotechnologies used, costs, and time-to-results. The testing strategies used in hospitals are rapidly evolving. A study from 2008 showed that greater than 90 percent of labs in the United States use enzyme immunoassay because it is fast, inexpensive, and technically easy to perform.[17] Just 3 years later, however, data showed that 43 percent of laboratories in the United States employed nucleic acid amplification tests (NAAT) (e.g., polymerase chain reaction [PCR]).[18]

Currently, diagnostic tests used in clinical settings to diagnose CDI include immunoassays such as enzyme immunoassays, enzyme-linked immunosorbent assays, immunochromatography assay, tests for *C. difficile* toxins, and amplification of *C. difficile* DNA, through means such as polymerase chain reaction and loop mediated isothermal amplification. Some diagnostic tests rely on two-step procedures, making use of sensitive, inexpensive, fast screening tests for the presence of the organism followed by a second test for toxins if the first step test is positive. Toxigenic culture and cell cytotoxicity neutralization assay are no longer clinical standard practice and are not universally available. However, given the rapid evolution of testing strategies, studies of diagnostic test performance often use toxigenic culture or cell cytotoxicity neutralization assay as the reference standard. Physicians may not always be sufficiently educated as to which diagnostic test is best to use, the operating characteristics of the tests employed in their practice setting, and the relatively low likelihood of a false negative result (e.g., evidence suggests retesting with the same test is common practice, yet not recommended).

Treatment for mild to moderate CDI is generally metronidazole, in part because of the concern that overuse of vancomycin may contribute to increasing pathogen resistance. Vancomycin is recommended for severe initial incident CDI.[19] However, both vancomycin and metronidazole have been implicated in increased frequency of vancomycin-resistant enterococci.[20] A new agent, fidaxomicin, was approved by the FDA in 2011 for treatment of CDI. CER No. 3 found that while fidaxomicin was not superior for the initial cure of CDI, recurrence was less frequent with fidaxomicin than with vancomycin. Measuring cure can also be challenging, as no specific consensus exists regarding symptom resolution, clearance of the organism, or recurrence of CDI.

Treatment for relapsed or recurrent CDI, however, is much more problematic. CDI recurs in 15-35 percent of patients with one previous episode and 33-65 percent of patients who have had more than two episodes.[21] Currently, clinicians choose from a number of antibiotics and dosing protocols and adjunctive treatments such as the use of antimicrobials, probiotics, toxin-binding agents, and immune-system enhancing agents.[22-24] The goal of most adjunctive treatments is to

reduce patient susceptibility to relapse or reinfection. Fecal microbiota transplantation (FMT) in particular has garnered significant clinical interest. FMT transfers fecal microbiota from a healthy individual to a CDI patient to restore a healthy gut microbiota.

Preventing the spread of *C. difficile* within institutional settings is dependent on staff compliance with national guidelines and standards[25] and locally determined hygiene protocols. Unfortunately, protocols for targeted hospital acquired infections are not always congruent. For example, the availability of alcohol hand rubs improved physician compliance and reduced Methicillin-resistant Staphylococcus aureus (MRSA) infections,[26] yet *C. difficile* produces spores that can withstand hostile environments and are resistant to alcohol hand rubs and other routine antiseptics. Spores may be best removed by hand washing. Other institutional prevention strategies may be required as *C. difficile* transmission knowledge develops. For example, one study isolated *C. difficile* spores from air samples in a UK hospital, 4 to 7 weeks after the last confirmed CDI case in the ward, and successfully cultured the bacterium.[27]

Community-acquired and community-onset CDI, where CDI occurs outside the institutional setting, complicates measuring the effectiveness of prevention within an institutional setting. The pathogenesis of CDI is complex and incompletely understood, and on-set may occur as late as several months after hospitalization or antibiotic use.

Several main findings were reported in CER No. 3. For diagnostic testing, direct comparisons of commercially available enzyme immunoassays for *C. difficile* toxins A and B did not find major differences in sensitivity or specificity. Limited evidence suggested that tests for genes related to *C. difficile* toxins production may be more sensitive than immunoassays, but that specificities were inconsistent. Moderate-strength evidence in favor of antibiotic restriction policies for prevention was found. While no antimicrobial was clearly superior for the initial cure of CDI, as noted above, recurrence was less frequent with fidaxomicin than with vancomycin. Numerous potential new forms of treatment were examined and fecal microbiota transplants for multiple recurrences appeared promising. However, with the numerous new publications identified in the surveillance report, an update of the review is merited.

In this update, we will systematically review and assess the evidence for diagnosis, prevention and treatment of *C. difficile* using the original report and newly available evidence. We will use essentially the same search strategy and review methodology, minimally updated to meet current review methods guidance. Some minor modifications to the key questions were made to focus the update on current clinical concerns and due to the scarce literature base. Specifically, we have deleted several subquestions regarding treatment effectiveness for subgroups. Since there has been some growth in the diagnostic testing literature, and diagnostic testing continues to be an area of decisional conflict, we also added a subquestion for testing strategy effects on final patient or health system outcomes.

II. The Key Questions

KQ1: How do different methods for detection of toxigenic *C. difficile* to assist with diagnosis of CDI compare in their sensitivity, specificity, and predictive values?
- a. Overall
- b. Do performance measures vary with sample characteristics?
- c. Does testing strategy impact patient health or health system outcomes?

KQ2: What are effective prevention strategies?
- a. What is the effectiveness of current prevention strategies?
- b. What are the harms associated with prevention strategies?

c. How sustainable are prevention practices in health care (outpatient, hospital inpatient, extended care) and community settings?

KQ3: What is the comparative effectiveness and harms of different antibiotic treatments?
   a. Does effectiveness vary by disease severity?

KQ4: What are the effectiveness and harms of nonantibiotic adjunctive interventions?
   a. Overall
   b. In patients with relapse/recurrent CDI.

Population, Interventions, Comparators, Outcomes, Timing, Settings (PICOTS)

Tables 1-3 provide the PICOTs by the key questions.

Table 1. Review PICOTS for KQ1 Diagnostics

PICOT	Included	Excluded
Population	Adults with clinical signs consistent with CDI	Pediatric patients alone Patients not suspected to have CDI; healthy patients
Intervention	Diagnostic tests for toxin producing *C. difficile*: • Immuoassays (EIAs, ELISA, immunochormatography assays) • Tests for toxins • Two step strategies • DNA amplification (PCR, LAMP)	Tests of stool culture alone. Tests to validate a technique in "known" or proven samples. Tests in which the reference standard is not applied to all samples. Tests examining cost characteristics. Tests not commercially available in the U.S. Tests only typing *C. difficile* strains. Tests establishing proof of concept for new testing techniques (such as fecal calprotectin)
Comparator groups	Reference Standard: cell cytotoxicity assay and/or toxigenic stool culture Comparators: any includable diagnostic test listed above as intervention. For health system and patient outcomes: historical data comparators may be used.	In-house laboratory tests not commercially available.
Outcomes	Sensitivity Specificity Predictive values Time-to-results Patient outcomes Health system outcomes	
Timing	Time to test results For patient or health system outcomes: no specific time requirement	
Setting	Healthcare facilities: outpatient, inpatient, extended care	

Table 2. Review PICOTS for KQ2 Prevention

PICOT	Included	Excluded
Population	Primary prevention: Adults at risk for CDI Recurrence prevention: Adults with clinical signs consistent with CDI	
Intervention	Antibiotic stewardship, education, bundled preventive programs, prebiotics or probiotics used as preventive measures Hospital inpatient environmental cleaning, monitoring, or surveillance	

PICOT	Included	Excluded
	Environmental cleaning for long-term care facilities	
Comparator groups	Usual prevention practices for prevention strategies	
Outcomes	CDI incidence rates CDI complication rates CDI mortality rates Harms, such as increase in organism resistance, hospital cleaning staff safety (bundled prevention programs), infection by introduced probiotics, isolation harms. Intermediate Outcomes • Appropriate antibiotic use. • Positive environmental cultures. • Days to resolution of symptoms (shorter window for transmission). • Other prevention strategy-related process variable demonstrating prevention strategy was taken up.	Studies that do not report CDI incidence rates and tie incidence to the intermediate process measures.
Timing	Variable	
Setting	Healthcare facilities: outpatient, inpatient, extended care.	

Table 3. Review PICOTS for KQ3 and KQ4 Treatments

PICOT	Included	Excluded
Population	Adults with clinical signs consistent with CDI	Pediatric, nonhuman, in vivo, or healthy volunteers.
Intervention	Standard antibiotic treatments: • Metronidazole • Rifaxamin • Vancomycin • Fidaxomicin Nonantibiotic adjunctive treatments: • Fecal transplant • Immunoglobulin • Pre/probiotics • Toxin binding agents • Rifampicin • Other new treatments available in the U.S.	Treatments approved outside of the U.S. that are not available in the U.S.
Comparator groups	Standard antibiotic treatments: active treatments such as metronidazole or vancomycin. Nonantibiotic adjunctive treatments: placebo, active controls, usual care.	
Outcomes	Mortality Recurrence (study author defined) Clearance (study author defined) Complications CDI-related colectomy rate Symptom resolution (study author defined) Harms, such as delayed treatment response	
Timing	Variable, generally from 4 weeks to several months	
Setting	Healthcare facilities: outpatient, inpatient, extended care	

III. Analytic Framework

Figure 1. Framework for diagnostic testing and treatment

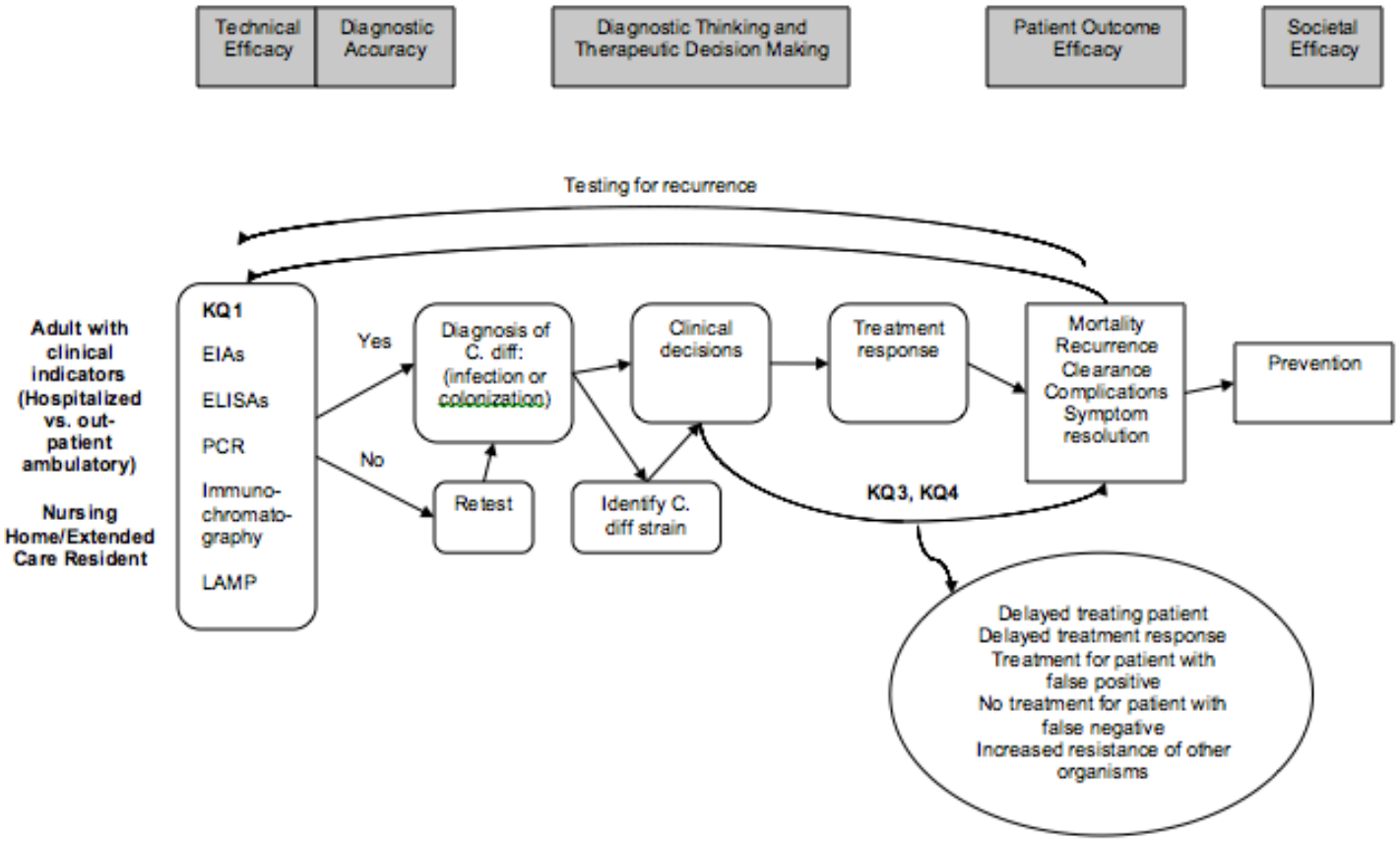
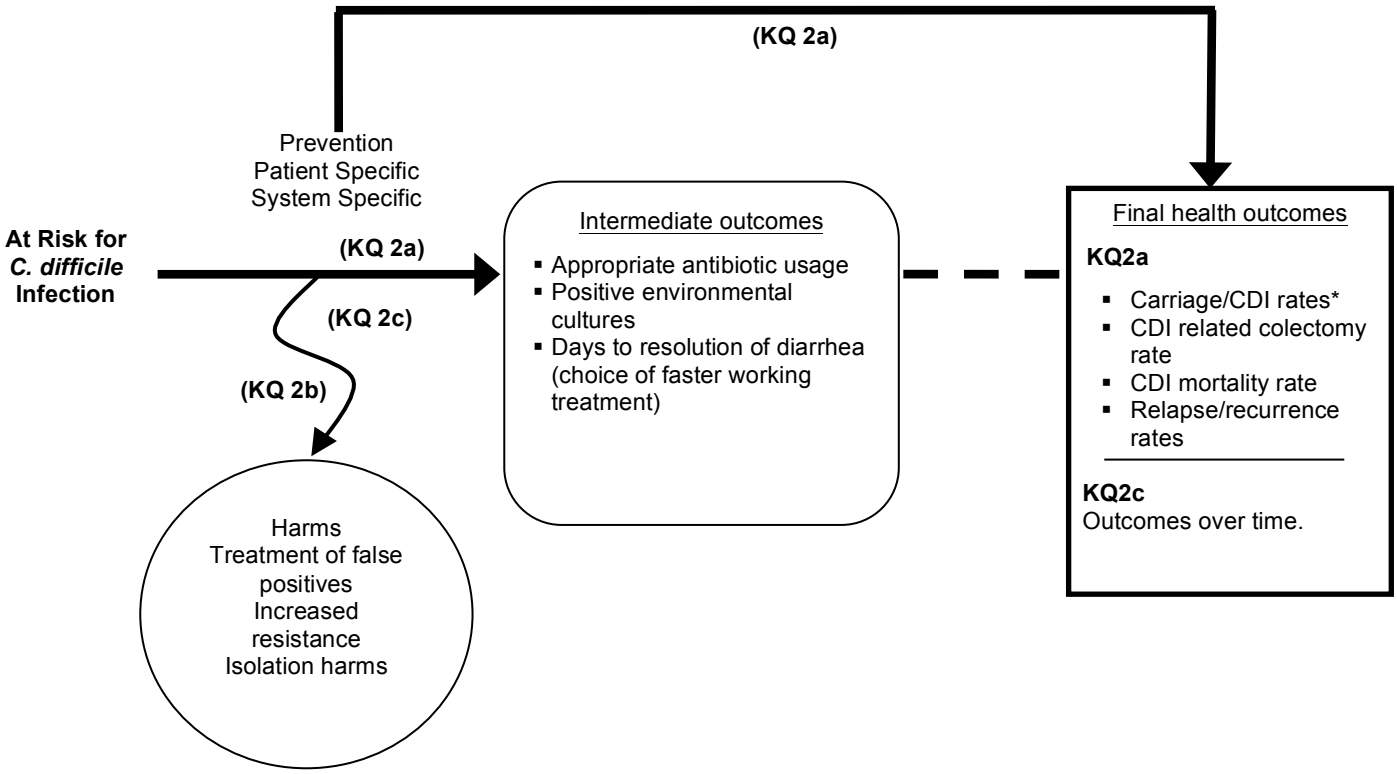


Figure 2. Analytic framework for CDI prevention



IV. Methods

A. Criteria for Inclusion/Exclusion of Studies in the Review

Studies will be included in the review based on the PICOTS framework outlined above and the study-specific inclusion criteria described in Table 4.

Table 4. Study inclusion criteria

Category	Criteria for Inclusion
Study Enrollment	Studies that enroll adults with suspected CDI
Study Design and Quality	Any: Systematic reviews with relevant questions of fair or good quality (see Section D below); must include risk of bias assessment with validated tools. Diagnosis: Studies of diagnostic accuracy assessing the operating characteristics of commercially available diagnostic test(s) for CDI in adult patients suspected of having CDI that include cell cytotoxicity neutralization assay (CCNA) or toxigenic culture as the reference standard applied to all samples. Prevention: RCTs, nonrandomized controlled trials, prospective cohort studies, retrospective cohort, time series, and before/after trials will be included. Cohort studies must include a comparator and appropriate methods to correct for selection bias. For Risk Factor studies, studies must be prospective with appropriate methods to correct for selection bias, the methods for the risk factor analysis were specified, the study included a clearly defined control group, for risk of CDI, not *C. difficile* colonization, CDI definition included diarrhea and a positive test for *C. difficile* toxin or PCR. Standard Treatment: RCTs, nonrandomized controlled trials, and prospective

Category	Criteria for Inclusion
	cohort studies will be included for each population and treatment option. Prospective studies must include a comparator and appropriate methods to correct for selection bias. Nonantibiotic standard treatment: RCTs, nonrandomized controlled trials, prospective cohort studies, and case series (at least 10 subjects) will be included for each population and treatment option. Prospective studies must include a comparator and appropriate methods to correct for selection bias. Studies specifically addressing treatment harms may also include retrospective and case series designs. Observational studies that do not adequately report study information to allow the abstraction of time sequences for treatment and followup duration or have indeterminable numerators and denominators for outcomes and adverse event rates will be excluded at the abstraction phase.
Time of Publication	Update from previous systematic review. We will scan 2011 forward to assure all published literature was identified.
Publication Type	Published in peer reviewed journals
Language of Publication	English

B. Searching for the Evidence: Literature Search Strategies for Identification of Relevant Studies to Answer the Key Questions

Our search methods will be essentially the same as were used for CER No. 3. We will search several databases, including Ovid MEDLINE, Cochrane Central Register of Controlled Trials (CENTRAL), and EMBASE from 2010 to the present to update CER No. 3. The keyword search for 'difficile' is highly specific yet sensitive to *C. difficile* related articles. We will use tested search strings to focus the search algorithms for randomized controlled trials (RCTs) and observational studies. Since diagnostic search filters with good sensitivity and specificity have not yet been established, a search algorithm without study-type filters will be used to screen for diagnostic studies. The search algorithms are provided in Appendix A.

We will review bibliographic database search results for studies relevant to our PICOTS framework and study-specific criteria. Search results will be downloaded to EndNote. Titles and abstracts will be reviewed by two independent investigators to identify studies meeting PICOTS framework and inclusion/exclusion criteria. All studies identified as relevant by either investigator will undergo full-text screening. We will track the number of non-English studies that appear eligible based upon English title and abstract to assess the magnitude of studies excluded for language. Two investigators will independently perform full-text screening to determine if inclusion criteria are met. Differences in screening decisions will be resolved by consultation between investigators, and, if necessary, consultation with a third investigator. We will document the inclusion and exclusion status of citations undergoing full-text screening. Throughout the screening process, team members will meet regularly to discuss training material and issues as they arise to ensure consistency of inclusion criteria application.

We will conduct additional grey literature searching to identify relevant completed and ongoing studies. Relevant grey literature resources include trial registries and funded research databases. We will search ClinicalTrials.gov and the International Controlled Trials Registry Platform (ICTRP) for ongoing studies. Scientific information packet (SIP)

letters and emails will be sent to identified relevant industry stakeholders requesting submission of published and unpublished information on their product(s). Grey literature search results will be used to identify studies, outcomes, and analyses not reported in the published literature to assess publication and reporting bias and inform future research needs.

We will update searches while the draft report is under public/peer review.

C. Data Abstraction and Data Management

Recent, relevant systematic reviews determined to have fair or good quality will be used to replace de novo extraction for specific population/treatment/outcome comparisons to which they apply, when feasible. Only systematic reviews that assessed and reported individual study risk of bias will be assessed for quality. For systematic reviews that do not meet all the feasibility criteria but are of fair or good quality, we may use their abstracted individual study, population, and outcome data, which will be verified by a trained abstractor. If a fair or good quality systematic review included both published and unpublished data, we will abstract only the published individual study data rather than the systematic review data. From systematic reviews, we will extract author, year of publication, literature search dates, eligibility criteria, relevant synthesis results, and strength of evidence assessment (see section F below). If an included systematic review does not assess strength of evidence for each outcome, we will use data provided by the systematic review to assess the strength of evidence. Studies included in the prior published systematic reviews will be tabled and compared to our search results for unique population-treatment-outcome comparisons to avoid double counting study results. Systematic reviews of fair or good quality that are deemed to have potential author conflict of interest, such as due to reviewing a body of literature to which the authors had substantially contributed, will be subjected to random quality checks of 10 percent of included study data abstraction. We will extract author, year of publication, eligibility criteria, individual study overall risk of bias (if review does not provide strength of evidence), and synthesis results.

For individual trials, one investigator will extract relevant study, population demographic, risk of bias elements, and outcomes data. Data fields will include all fields provided in the original CER, including author, year of publication; setting, subject inclusion and exclusion criteria, intervention and control characteristics (intervention components, timing, frequency, and duration), followup duration, participant baseline demographics, method of diagnosis, enrollment, and severity, descriptions and results of primary outcomes and adverse effects, and study funding source. Relevant data will be extracted into web-based extraction forms created in Microsoft Excel. Evidence tables will be reviewed and verified for accuracy by a second investigator.

D. Assessment of Methodological Risk of Bias of Individual Studies

Risk of bias of eligible studies will be assessed using instruments specific to study design. For diagnostic studies, we will use the QUADAS 2 tool.[28] Risk of bias domains include patient selection, index tests, reference standards, and test flow and timing. Specific study methodology or conduct will be used to judge potential risk of bias with respect to each domain following guidance in the Methods Guide for Medical Test Reviews.[29]

Our selection criteria are consistent with three of the four quality domains delineated in QUADAS-2 (index test, reference test, and flow and timing). In addition, we are including studies that are conducted in patients at risk for CDI. This selection strategy, with its focus on strong study design, means we do not expect a significant number of included studies to have high risk of bias. Differences between medium and high risk of bias will be determined by nuances in study conduct across the four domains.

For RCTs, questionnaires developed from the Cochrane Risk of Bias tool will be used. The seven domains included in this tool include sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data (i.e., was incomplete outcome data adequately addressed), selective reporting, and other sources of bias (i.e., problems not covered by other domains). Study power will be assessed in 'other sources of bias' in studies with data that are not eligible for pooling. Specific study methodology or conduct will be used to judge potential risk of bias with respect to each domain following guidance in the Cochrane Handbook for Systematic Reviews of Interventions, Version 5.1.0.[30,31]

We developed an instrument for assessing risk of bias for observational studies based on the RTI Observational Studies Risk of Bias and Precision Item Bank.[32] (Appendix B) We selected items most relevant in assessing risk of bias for this topic, including participant selection, attrition, ascertainment, and appropriateness of analytic methods. Study power will be assessed in 'other sources of bias' in studies with data that are not eligible for pooling. The form will be tested by investigators using an initial sample of included studies and will be finalized by full team input.

Systematic review quality will be assessed using modified AMSTAR criteria.[33] An additional question will be added to assess the coherence of the review findings and conclusions. Systematic reviews' risk of bias and strength of evidence methods must meet accepted AHRQ EPC standards (such as Cochrane review methods or GRADE). Since the use of a validated risk of bias tool is an inclusion criterion, we anticipate consistency across risk of bias approaches. However, if reviews used different tools to assess risk of bias in individual studies, we will determine if the main elements that address sources of potential bias were covered by their assessment/tool. If the risk of bias assessment tool is similar to our approach, we will separately assess risk of bias on only a sample of primary studies from prior reviews. If the tool is not similar or misses important potential bias elements, we will reassess risk of bias using our approach.

Two investigators will independently assess risk of bias for all included studies. Investigators will consult to reconcile any discrepancies in overall risk of bias assessments. Overall summary risk of bias assessments for each study will be classified as low, moderate, or high based upon the collective risk of bias inherent in each domain and confidence that the results are believable given the study's limitations. When the two investigators disagree, a third party will be consulted to reconcile the summary judgment.

E. Data Synthesis -

We will perform the same analyses as in the original CER, reanalyzing meta-analyses where new relevant studies have been identified. Evidence and summary tables will follow those used for CER No. 3 wherever possible. Information from individual studies reviewed in CER No. 3 will be brought forward into the update report when meta-analysis is performed using such information. Otherwise, tables will provide studies

identified for the update and text will note if and how overall results from CER No. 3 are amended. Data will be analyzed in RevMan 5.21 software.[34]

When a comparison has been adequately addressed by a previous systematic review of acceptable quality (fair or high quality according to modified AMSTAR) and no new studies are available, we will reiterate the conclusions drawn from that review. When new trials are available, previous systematic review data will be synthesized with data from the additional trials, when possible. If we identify a substantial number of new studies not included in the original review, we may opt to create a new study pool for re-analysis. We will analyze included studies in these systematic reviews to assess the balance of publication dates and study-level risk of bias relative to the original research we will include.

We will summarize included study characteristics and outcomes in evidence tables. We will emphasize patient-centered outcomes in the evidence synthesis. We will attempt to identify established minimum important differences (MIDs) for key outcomes measurement instruments using targeted literature searches of instruments identified in targeted literature searches and Technical Expert Panel input. We will try to use MIDs to assess the efficacy and comparative effectiveness of outcomes with well-established MIDs, but many of our outcomes are not likely to have established MIDs. When standard MIDs for particular outcomes are not available, we will use statistical differences to assess efficacy and comparative effectiveness and calculate the minimum detectable difference that the data allowed (β=.8, α=.05).

For diagnostic studies we will look at the reference standards and base contrasts on the type of reference standard and respective operating characteristics.[35,36] We will focus on the differences between test category/methodology sensitivities and specificities rather than on specific test sensitivities and specificities themselves. We expect to pool one-step NAAT studies using random effects models; two-step studies that include NAAT tests (likely PCR) will be pooled with other two-step processes. Since two primary endpoints will be used, sensitivity and specificity, we will calculate 99 percent confidence intervals (CI). Depending on the heterogeneity of prevalence of CDI in diagnostic accuracy studies, we may also calculate positive and negative predictive values of the diagnostic tests for *C. Difficile*. Further, when possible, we will calculate positive and negative likelihood ratios that allow clinicians to easily modify pre-test probability of CDI based on test results.[37]

For studies that use multiple reference standards, such as culture, toxigenic culture, and cell cytotoxicity neutralization assay, we will use toxigenic culture as the reference standard. If different reference standards are used for specific subgroups (such as study site) and no one is used across all the samples, then we will use the reference standard that was used in interpretation of the index test.

For prevention and treatment studies, if certain comparisons can be pooled, we will meta-analyze the data using a random effects model. We will calculate risk ratios (RR) and absolute risk differences (RD) with the corresponding 95 percent CI for binary primary outcomes. Weighted mean differences (WMD) and/or standardized mean differences (SMD) with the corresponding 95 percent CIs will be calculated for continuous outcomes. We will assess the clinical and methodological heterogeneity and variation in effect size to determine appropriateness of pooling data.[38] We will assess statistical heterogeneity with Cochran's Q test and measure magnitude with I^2 statistic.

We will assess harms as dichotomous variables to acknowledge the inherent difficulties of assessing harms and also to simplify analysis. There are various ways to assess harms; each has problems. One can use RCT and controlled cohort data, but they generally have small samples and short followups. One can use case series, but they have no controls and the rate of "adverse events" among placebo groups is high.[39] One can use case-control studies, but they are subject to recall bias. One can examine the general experience with the intervention, but this does not exclude the possibility that persons with the target condition have different susceptibilities. We will use reported harms from RCTs, prospective cohort, retrospective case-control, and case series.

F. Grading the Strength of Evidence (SOE) for Major Comparisons and Outcomes

The overall strength of evidence for primary outcomes of KQ1 within each comparison will be evaluated based on five required domains: 1) study limitations (risk of bias); 2) directness (single, direct link between intervention and outcome); 3) consistency (similarity of effect direction and size); 4) precision (degree of certainty around an estimate); and 5) reporting bias.[40] Based on study design and risk of bias, study limitations will be rated as low, medium, or high. Consistency will be rated as consistent, inconsistent, or unknown/not applicable (e.g., single study) based on the whether intervention effects are similar in direction and magnitude, and statistical significance of all studies. Directness will be rated as either direct or indirect based on the need for indirect comparisons when inference requires observations across studies. That is, more than one step is needed to reach the conclusion. Precision will be rated as precise or imprecise based on the degree of certainty surrounding each effect estimate or qualitative finding. An imprecise estimate is one for which the confidence interval is wide enough to include clinically distinct conclusions. For outcomes found to have at least moderate or high strength of evidence, reporting bias will be evaluated by the potential for publication bias, selective outcome reporting bias, and selective analysis reporting bias by comparing reported results with those mentioned in the methods section and an assessment of the grey literature to assess potentially unpublished studies. Other factors that may be considered in assessing strength of evidence include dose-response relationship, the presence of confounders, and strength of association.

Based on these factors, the overall strength of evidence for each outcome will be rated as.[40]

- **High:** Very confident that estimate of effect lies close to true effect. Few or no deficiencies in body of evidence, findings believed to be stable.
- **Moderate:** Moderately confident that estimate of effect lies close to true effect. Some deficiencies in body of evidence; findings likely to be stable, but some doubt.
- **Low:** Limited confidence that estimate of effect lies close to true effect; major or numerous deficiencies in body of evidence. Additional evidence necessary before concluding that findings are stable or that estimate of effect is close to true effect.
- **Insufficient:** No evidence, unable to estimate an effect, or no confidence in estimate of effect. No evidence is available or the body of evidence precludes judgment.

We will assess strength of evidence for published systematic reviews replacing de novo review processes that did not provide a strength of evidence assessment based on a GRADE or GRADE-equivalent method and incorporating all relevant articles, including new articles identified in bridge searches. For prior systematic reviews that did provide acceptable strength of evidence, the impact of new articles on the overall body of evidence will take into consideration the differences in strength of evidence domains and the relative contributions of the prior review and the new articles.

G. Assessing Applicability

Applicability of studies will be determined according to the PICOTS framework. Study characteristics that may affect applicability include, but are not limited to, the population from which the study participants are enrolled, diagnostic assessment processes, narrow eligibility criteria, and patient and intervention characteristics different from those described by population studies of *C. difficile*.[41]

Applicability of studies of diagnostic accuracy of diagnostic tests for CDI may be influenced by the selection of patient samples included in the studies included and the degree (if any) of delineation of the demographic and clinical characteristics of the studies' respective patient populations and how these characteristics compare with a local population. Further, certain diagnostic tests may not be available to all clinicians depending on local health system factors.

V. References

1. Butler M, Bliss D, Drekonja D, et al. Effectiveness of Early Diagnosis, Prevention, and Treatment of Clostridium difficile Infection. Rockville, MD: Prepared by the Minnesota Evidence-based Practice Center for the Agency for Healthcare Research and Quality under Contract No. 290-02-0009; Comparative Effectiveness Review Number 31, AHRQ Publication No. 11(12)-EHC051-EF. December 2011. http://www.effectivehealthcare.ahrq.gov/ehc/products/115/772/CER-31_Cdiff_20111220.pdf.
2. Butler M, Bliss D, Drekonja D, et al. Effectiveness of Early Diagnosis, Prevention, and Treatment of *Clostridium difficile* Infection--Nomination Summary Document. Agency for Healthcare Research and Quality. Available at: http://effectivehealthcare.ahrq.gov/ehc/assets/File/c-diff-infections-nomination-140925.pdf. Accessed Nov 4, 2014.
3. Murphy SL, Xu J, Kochanek KD. Deaths: final data for 2010. Natl Vital Stat Rep 2013; May 8;61(4):1-117. PMID: 24979972.
4. Hoyert DL, Xu J. Deaths: preliminary data for 2011. Natl Vital Stat Rep 2012; Oct 10;61(6):1-51. PMID: 24984457.
5. Hebuterne X. Gut changes attributed to ageing: effects on intestinal microflora. Current opinion in clinical nutrition and metabolic care 2003; Jan;6(1):49-54. PMID: 12496680.
6. Kim J, Smathers SA, Prasad P, et al. Epidemiological features of Clostridium difficile-associated disease among inpatients at children's hospitals in the United States, 2001-2006. Pediatrics 2008; Dec;122(6):1266-70. PMID: 19047244.
7. Campbell RJ, Giljahn L, Machesky K, et al. Clostridium difficile infection in Ohio hospitals and nursing homes during 2006. Infect Control Hosp Epidemiol 2009; Jun;30(6):526-33. PMID: 19419272.
8. McFarland LV. Renewed interest in a difficult disease: Clostridium difficile infections--epidemiology and current treatment strategies. Curr 2009; Jan;25(1):24-35. PMID: 19114771.
9. Jarvis WR, Schlosser J, Jarvis AA, et al. National point prevalence of Clostridium difficile in US health care facility inpatients, 2008. American journal of infection control 2009; May;37(4):263-70. PMID: 19278754.
10. Severe Clostridium difficile-associated disease in populations previously at low

risk--four states, 2005. Mmwr 2005; Dec 2;54(47):1201-5. PMID: 16319813.

11. Hubert B, Loo VG, Bourgault AM, et al. A portrait of the geographic dissemination of the Clostridium difficile North American pulsed-field type 1 strain and the epidemiology of C. difficile-associated disease in Quebec. Clin Infect Dis 2007; Jan 15;44(2):238-44. PMID: 17173224.
12. Karpa KD. Probiotics for Clostridium difficile diarrhea: putting it into perspective. The Annals of pharmacotherapy 2007; Jul;41(7):1284-7. PMID: 17595302.
13. Owens RC, Jr., Donskey CJ, Gaynes RP, et al. Antimicrobial-associated risk factors for Clostridium difficile infection. Clinical Infectious Diseases 2008; Jan 15;46(Suppl 1):S19-31. PMID: 3566.
14. Abou Chakra C, Pepin J, Sirard S, et al. Risk Factors for recurrence, complications and mortality in Clostridium difficile infection: a systematic review. PLoS ONE 2014; 9(6):e98400. PMID.
15. Garey KW, Sethi S, Yadav Y, et al. Meta-analysis to assess risk factors for recurrent Clostridium difficile infection. Journal of Hospital Infection 2008; Dec;70(4):298-304. PMID: 2914.
16. Starr JM, Campbell A, Renshaw E, et al. Spatio-temporal stochastic modelling of Clostridium difficile. The Journal of hospital infection 2009; Jan;71(1):49-56. PMID: 19013677.
17. Bartlett JG, Gerding DN. Clinical recognition and diagnosis of Clostridium difficile infection. Clin Infect Dis 2008; Jan 15;46 Suppl 1:S12-8. PMID: 18177217.
18. Cohen J, Limbago B, Dumyati G, et al. Impact of changes in clostridium difficile testing practices on stool rejection policies and C. difficile positivity rates across multiple laboratories in the United States. J. Clin. Microbiol. 2014; 52(2):632-4. PMID: 24478500.
19. Surawicz CM, Brandt LJ, Binion DG, et al. Guidelines for diagnosis, treatment, and prevention of *Clostridium difficile* infections. The American journal of gastroenterology 2013; 108:478-98. PMID.
20. Pepin J. Vancomycin for the treatment of Clostridium difficile Infection: for whom is this expensive bullet really magic? Clinical Infectious Diseases 2008; May 15;46(10):1493-8. PMID: 18419481.
21. Gerding DN, Muto CA, Owens RC, Jr. Treatment of Clostridium difficile infection. Clinical Infectious Diseases 2008; Jan 15;46 Suppl 1:S32-42. PMID: 18177219.
22. Halsey J. Current and future treatment modalities for Clostridium difficile-associated disease. Am J Health-Syst Pharm 2008; Apr 15;65(8):705-15. PMID: 18387898.
23. Jodlowski TZ, Oehler R, Kam LW, et al. Emerging therapies in the treatment of Clostridium difficile-associated disease. Ann Pharmacother 2006; Dec;40(12):2164-9. PMID: 17119105.
24. Stepan C, Surawicz CM. Treatment strategies for C. difficile associated diarrhea. Acta Gastroenterol Latinoam 2007; Sep;37(3):183-91. PMID: 17955730.
25. Dubberke ER, Gerding DN, Classen D, et al. Strategies to prevent clostridium difficile infections in acute care hospitals. Infect Control Hosp Epidemiol 2008; Oct;29 Suppl 1:S81-92. PMID: 18840091.
26. Gordin FM, Schultz ME, Huber RA, et al. Reduction in nosocomial transmission of drug-resistant bacteria after introduction of an alcohol-based handrub. Infect Control Hosp Epidemiol 2005; Jul;26(7):650-3. PMID: 16092747.
27. Roberts K, Smith CF, Snelling AM, et al. Aerial dissemination of Clostridium difficile spores. BMC Infectious Diseases 2008; 8:7. PMID: 65.
28. Whiting P, Rutjes A, Westwood M, et al. QUADAS-2: A revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 2011; 155(8):529-36. PMID.
29. Santaguida PL, Riley CM, Matchar DB. Chapter 5: assessing risk of bias as a domain of quality in medical test studies. J Gen Intern Med 2012; Jun;27 Suppl 1:S33-8. PMID: 22648673.
30. Higgins JPT, Altman D, Sterne J. Chapter 8: Assessing risk of bias in included studies. In: Higgins JPT, Green S, eds. Cochrane Handbook for Systematic Reviews of Interventions: Version 5.1.0: The Cochrane Collaboration; 2011.
31. Higgins JPT, Altman D, J. S. Chapter 8: Assessing risk of bias in included studies.

In: Higgins JPT GS, ed. Cochrane Handbook for Systematic Reviews of Interventions: Version 5.1.0. Repr. ed: The Cochrane Collaboration; 2011.

32. Viswanathan M, Ansari M, Berkman N, et al. Assessing the Risk of Bias of Individual Studies in Systematic Reviews of Health Care Interventions: AHRQ. 2012.
33. White C, Ip S, McPheeters M, et al. Using existing systematic reviews to replace de novo processes in conducting Comparative Effectiveness Reviews. Rockville, MD: Agency for Healthcare Research and Quality. 2009. http://effectivehealthcare.ahrq.gov/repFiles/methodsguide/systematicreviewsreplacedenovo.pdf.
34. [computer program]. Version.
35. Trikalinos TA, Balion CM. Chapter 9: options for summarizing medical test performance in the absence of a "gold standard". J Gen Intern Med 2012; Jun;27 Suppl 1:S67-75. PMID: 22648677.
36. Trikalinos TA, Balion CM, Coleman CI, et al. Chapter 8: meta-analysis of test performance when there is a "gold standard". J Gen Intern Med 2012; Jun;27 Suppl 1:S56-66. PMID: 22648676.
37. Sackett D. A primer on the precision and accuracy of the clinical examination. JAMA 1992; 267(19):2638-44. PMID.
38. Fu R, Gartlehner G, Grant M, et al. Conducting quantitative synthesis when comparing medical interventions: AHRQ and the Effective Health Care Program. J Clin Epidemiol 2011; Nov;64(11):1187-97. PMID: 21477993.
39. Weihrauch TR, Gauler TC. Placebo--efficacy and adverse effects in controlled clinical trials. Arzneimittelforschung 1999; May;49(5):385-93. PMID: 10367099.
40. Berkman ND, Lohr K, Ansari M, et al. Grading the strength of a body of evidence when assessing health care interventions for the effective health care program of the Agency for Healthcare Research and Quality: An update. 290-2007-10056-I PbtR-UE-bPCuCN, trans. Methods Guide for Comparative Effectiveness Reviews Vol AHRQ Publication No. 13(14)-EHC 130-EF. November ed. Rockville, MD: Agency for Healthcare Research and Quality; 2013.
41. Atkins D, Chang SM, Gartlehner G, et al. Assessing applicability when comparing medical interventions: AHRQ and the Effective Health Care Program. J Clin Epidemiol 2011; Nov;64(11):1198-207. PMID: 21463926.

VI. Definition of Terms

AHRQ	Agency for Healthcare Research and Quality
CDI	Clostridium difficile infection
CENTRAL	Cochrane Central Register of Controlled Trials
CER	Comparative Effectiveness Review
CI	Confidence interval
FMT	Fecal microbiota transplantation
ICTRP	International Controlled Trials Registry Platform
MID	Minimum important difference
MRSA	Methicillin-resistant Staphylococcus aureus
NAAT	Nucleic acid amplification tests
PCR	Polymerase chain reaction
PICOTS	Population, Interventions, Comparators, Outcomes, Timing, Settings
RCT	Randomized controlled trial
RD	Risk difference
RevMan	Review Manager
RR	Risk ratio
SIP	Scientific information packet
SMD	Standardized mean difference

SOE Strength of evidence
WMD Weighted mean difference

VII. Summary of Protocol Amendments

If we need to amend this protocol, we will give the date of each amendment, describe the change and give the rationale in this section. Changes will not be incorporated into the protocol. Example table below:

Date	Section	Original Protocol	Revised Protocol	Rationale

VIII. Review of Key Questions

AHRQ posted the key questions on the Effective Health Care Web site for public comment. The EPC refined and finalized the key questions after review of the public comments and input from Key Informants and the Technical Expert Panel. This input is intended to ensure that the key questions are specific and relevant.

IX. Key Informants

Key Informants are the end users of research, including patients and caregivers, practicing clinicians, relevant professional and consumer organizations, purchasers of health care, and others with experience in making health care decisions. Within the EPC program, the Key Informant role is to provide input into identifying the Key Questions for research that will inform healthcare decisions. The EPC solicits input from Key Informants when developing questions for systematic review or when identifying high priority research gaps and needed new research. Key Informants are not involved in analyzing the evidence or writing the report and have not reviewed the report, except as given the opportunity to do so through the peer or public review mechanism.

Key Informants must disclose any financial conflicts of interest greater than $10,000 and any other relevant business or professional conflicts of interest. Because of their role as end-users, individuals are invited to serve as Key Informants and those who present with potential conflicts may be retained. The Task Order Officer and the EPC work to balance, manage, or mitigate any potential conflicts of interest identified.

X. Technical Experts

Technical Experts constitute a multidisciplinary group of clinical, content, and methodological experts who provide input in defining populations, interventions, comparisons, or outcomes and identify particular studies or databases to search. They are selected to provide broad expertise and perspectives specific to the topic under development. Divergent and conflicting opinions are common and perceived as health scientific discourse that results in a thoughtful, relevant systematic review. Therefore, study questions, design, and methodological approaches do not necessarily represent the views of individual technical and content experts. Technical Experts provide information

to the EPC to identify literature search strategies and recommend approaches to specific issues as requested by the EPC. Technical Experts do not do analysis of any kind nor do they contribute to the writing of the report. They have not reviewed the report, except as given the opportunity to do so through the peer or public review mechanism.

Technical Experts must disclose any financial conflicts of interest greater than $10,000 and any other relevant business or professional conflicts of interest. Because of their unique clinical or content expertise, individuals are invited to serve as Technical Experts and those who present with potential conflicts may be retained. The Task Order Officer and the EPC work to balance, manage, or mitigate any potential conflicts of interest identified.

XI. Peer Reviewers

Peer reviewers are invited to provide written comments on the draft report based on their clinical, content, or methodological expertise. The EPC considers all peer review comments on the draft report in preparation of the final report. Peer reviewers do not participate in writing or editing of the final report or other products. The final report does not necessarily represent the views of individual reviewers. The EPC will complete a disposition of all peer review comments. The disposition of comments for systematic reviews and technical briefs will be published 3 months after the publication of the evidence report.

Potential Peer Reviewers must disclose any financial conflicts of interest greater than $10,000 and any other relevant business or professional conflicts of interest. Invited Peer Reviewers may not have any financial conflict of interest greater than $10,000. Peer reviewers who disclose potential business or professional conflicts of interest may submit comments on draft reports through the public comment mechanism.

XII. EPC Team Disclosures

EPC core team members must disclose any financial conflicts of interest greater than $1,000 and any other relevant business or professional conflicts of interest. Related financial conflicts of interest that cumulatively total greater than $1,000 will usually disqualify EPC core team investigators.

XIII. Role of the Funder

This project was funded under Contract No. 290-20-1200016I from the Agency for Healthcare Research and Quality, U.S. Department of Health and Human Services. The Task Order Officer reviewed contract deliverables for adherence to contract requirements and quality. The authors of this report are responsible for its content. Statements in the report should not be construed as endorsement by the Agency for Healthcare Research and Quality or the U.S. Department of Health and Human Services.

Appendix A

Search String for C Difficile (general)

Database: Ovid MEDLINE
Search Strategy:
---

1 difficile.mp.
2 limit 1 to (english language and humans)
3 limit 2 to ("all adult (19 plus years)" or "young adult (19 to 24 years)" or "adult (19 to 44 years)" or "young adult and adult (19-24 and 19-44)" or "middle age (45 to 64 years)" or "middle aged (45 plus years)" or "all aged (65 and over)" or "aged (80 and over)")
4 randomized controlled trial.pt.
5 controlled clinical trial.pt.
6 randomized.ab.
7 placebo.ab.
8 drug therapy.fs.
9 randomly.ab.
10 trial.ab.
11 groups.ab.
12 or/4-11
13 (animals not (humans and animals)).sh.
14 12 not 13
15 3 and 14
16 limit 15 to (addresses or bibliography or biography or dictionary or directory or duplicate publication or editorial or interview or introductory journal article or lectures or legal cases or legislation or letter or news or newspaper article or patient education handout or portraits)
17 15 not 16
18 Cohort studies/or comparative study/ or followup studies/or prospective studies/or risk factors/or cohort.mp. or compared.mp. or groups.mp. or multivariate.mp.
19 limit 18 to (comment or editorial or historical article or interview or letter)
20 18 not 19
21 3 and 20
22 17 or 21

Search String for Diagnostics (not filtered for study design)

1 difficile.mp.
2 limit 1 to (english language and humans)
3 (animals not (humans and animals)).sh.
4. 2 not 3
5 limit 4 to (addresses or bibliography or biography or dictionary or directory or duplicate publication or editorial or interview or introductory journal article or lectures or legal cases or legislation or letter or news or newspaper article or patient education handout or portraits)
6. 4 not 5

Appendix B
Risk of Bias Assessment for Observational Studies

Question	Response	Criteria	Justification
	Internal Validity		
1. Study design: prospective, retrospective or mixed?	Prospective ☐	Outcome had not occurred when study was initiated; information was collected over time	
	Mixed ☐	One group was studied prospectively; other(s) retrospectively	
	Retrospective ☐	Analyzed data from past records, claims	
2. Were inclusion/exclusion criteria clearly stated?	Yes ☐	Clearly stated	
	Partially ☐	Some, but not all criteria stated or some not clearly stated.	
	No ☐	Unclear	
3. Were baseline characteristics measured using valid and reliable measures and are they equivalent in both groups?	Yes ☐	Valid measures, groups ~equivalent	
	No ☐	Non-validated measures or nonequivalent groups	
	Uncertain ☐	Could not be ascertained	
4. Were important variables known to impact the outcome(s) assessed at baseline?	Yes ☐	Yes, most or all known factors were assessed	
	No ☐	Critical factors are missing	
	Uncertain ☐		
5. Is the level of detail describing the intervention adequate?	Yes ☐	Intervention sufficiently described	
	Partially ☐	Some of the above features.	
	No ☐	Intervention poorly described	
6. Is the selection of the comparison group appropriate?	Yes ☐	Other adults with fecal incontinence with similar etiologic, demographic, severity and comorbid features	
7. Was the impact of a concurrent intervention or an unintended exposure that might bias results isolated?	Yes ☐	By inclusion criteria, protocol or other means	
	Partially ☐	Some were isolated, others were not	
	No ☐	Important concurrent interventions were not isolated or prohibited	
8. Were there attempts to balance the allocation across groups? (e.g., stratification, matching or propensity scores)	Yes ☐	(*If yes, what method was used?)*	
	No ☐		
	Uncertain ☐	Could not be ascertained	
9. Were outcomes assessors blinded?	Yes ☐	Who assessed outcomes?	
	No ☐		
	Uncertain ☐	Not reported	
10. Were outcomes assessed using valid and reliable measures, and used consistently across all study participants?	Yes ☐	Measures were valid and reliable (i.e., objective measure, validated scale/tool); consistent across groups	
	Partially ☐	Some of the above features	
	No ☐	None of the above features	
	Uncertain ☐	Could not be ascertained.	
11. Was length of followup the same for	Yes ☐		
	No ☐		

Question	Response	Criteria	Justification
		Internal Validity	
all groups?	Uncertain ☐	Could not be ascertained	
12. Did attrition result in differences in group characteristics between baseline and followup?	Yes ☐	*(If yes, for which followup period(s)?)*	
	No ☐		
	Uncertain ☐	Could not be ascertained	
13. If dissimilar baseline characteristics, does the analysis control for baseline differences between groups?	Yes ☐	What method?	
	No ☐		
	Uncertain ☐	Could not be ascertained	
14. Were confounding and/or effect modifying variables assessed using valid and reliable measures across all study participants?	Yes ☐		
	No ☐		
	Uncertain ☐	Could not be ascertained (i.e., retrospective designs where eligible at baseline could not be determined)	
	NA ☐	No confounders or effect modifiers included in the study.	
15. Were important confounding and effect modifying variables taken into account in design and/or analysis? (e.g., matching, stratification, interaction terms, multivariate analysis, or other statistical adjustment)	Yes ☐		
	Partially ☐	Some variables taken into account or adjustment achieved to some extent.	
	No ☐	Not accounted for or not identified.	
	Uncertain ☐	Could not be ascertained	
16. Are statistical methods used to assess the primary outcome appropriate to the data?	Yes ☐	Statistical techniques used must be appropriate to the data.	
	Partially ☐		
	No ☐		
	Uncertain ☐	Could not be ascertained	
17. Is there suggestion of selective outcome reporting?	Yes ☐		
	No ☐	Not all prespecified outcomes reported, subscales not prespecified reported, outcomes reported incompletely	
	Uncertain ☐	Could not be ascertained	
18. Was the funding source identified?	No ☐		
	Yes ☐	Who provided funding?	
	Uncertain ☐		
Question	**Response**	**Criteria**	**Justification**
		Internal Validity	
Overall Assessment			
Overall Risk of Bias assessment	Low ☐	Results are believable taking study limitations into consideration	
	Moderate ☐	Results are probably believable taking study limitations into consideration	
	High ☐	Results are uncertain taking study limitations into consideration	